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Effects of Laboratory Rearing on Gypsy Moth (Lepidoptera: Lymantriidae)

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Abstract

The New Jersey Standard Strain (NJSS) accounts for about 90 percent of the laboratory-reared gypsy moth, *Lymantria dispar* (L.), used for research and development in the United States. The history and performance of NJSS since its establishment in the laboratory in 1967 are reviewed and phenotypic changes in NJSS during and after 35 generations of domestication are defined. Variability in life history traits are compared with a near wild strain, and laboratory and field studies comparing behavior, susceptibility to microbials, and response to host-plant chemicals are reviewed. Phenotypic variability for most developmental traits have decreased in NJSS-FS reared by the USDA Forest Service. Generally, artificial selection, either intentional or as a result of rearing changes to increase production efficiency, has made NJSS-FS a faster developing, heavier, more fecund strain than wild strains. Use of NJSS for research and development should be assessed carefully relative to the effects of domestication and the need to equate the performance of NJSS to a specific research/development objective.

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Introduction

The gypsy moth, *Lymantria dispar,* is one of the most prolific forest defoliators in the Unites States. Since the introduction and establishment of this forest pest in Medford, Massachusetts, in 1868, the area of general infestation has expanded and now includes parts of Maine, Virginia, Ohio, and Michigan. Periodic infestations also have occurred as far south as South Carolina and to the west in California, Utah, Washington, and Oregon. Research and development of modern pest management strategies for controlling gypsy moth have depended on suitable laboratory techniques to establish and produce gypsy moth colonies in the laboratory year round (see ODell et al. 1984 for review).

Approximately 90 percent of the gypsy moth used for research and development in the United States are produced in two U.S. Department of Agriculture (USDA) insect-rearing facilities. These are located at the Animal and Plant Health Inspection Service's Otis Methods Development Center (APHIS-OMDC), Otis Air National Guard Base, Cape Cod, Massachusetts, and the Forest Service's Northeastern Center for Forest Health Research (CFHR), Northeastern Forest Experiment Station, Hamden, Connecticut. Both facilities use the New Jersey Standard Strain (NJSS) for production of sterile adults, F_1-sterile egg masses, and nuclear polyhedrosis virus (NPV), and for insects used in research by Federal, state, university, and industry scientists.

The establishment of colonies and adaptation of insects to laboratory conditions results in phenotypic and genetic changes with unknown effects on the performance of the insect. The potential genetic effects of laboratory domestication have been reviewed by Bartlett (1984, 1985). Bartlett (1984) concluded that "changes in genetic variability during domestication of an insect population will be unavoidable," but suggests that "those genetic changes need not ruin the program." Our intent here is to quantify phenotypic and genetic changes that have occurred in a gypsy moth population reared artificially in the laboratory for 36 generations. If genetic changes have occurred and appear to be correlated with reduced performance of the laboratory strain relative to a specific production objective (i.e., sterile male, NPV, research), then correction can be made by modifying the rearing process or increasing the genetic variability of the colony (Joslyn 1984).

Life History

Under natural conditions, the gypsy moth is univoltine. Eggs are laid in light brown setae-covered masses. The timing of oviposition varies with the normal progression of seasons in different geographic areas. In the Northeastern United States, eggs are laid in July and early August. Embryonation is completed in approximately 3 weeks at which time the insect enters dormancy, characterized by an aestival-autumnal-hibernal diapause in the fully developed larva within the egg (Tauber et al. 1990). Exposure to cold ($\leq$ 10°C) following embryonation hastens diapause, whereas warm conditions retard diapause development (Tauber et al. 1990). In the laboratory, the optimum period of cold exposure is 150 days (reviewed by Giese and Cittadino 1977). This allows shortening of the egg stage to 170 to 180 days under laboratory conditions. In the Northeast, egg hatch occurs in late April or early May, usually in synchrony with budbreak of the red oak (*Quercus rubra* L.), the major host of the gypsy moth in that region.

The gypsy moth larva has considerable plasticity in number of instars; generally, males have five instars and females six. One additional instar is common for both sexes (Leonard 1981) and we have observed as many as 11 in laboratory rearing. Larvae range in size from approximately 3 mm in the first stage to 50 to 90 mm in the sixth (Leonard 1981). Under natural conditions, pupation occurs in 50 to 60 days, with adult eclosion 10 to 15 days later. In the laboratory, at 25°C, the larval period is 28 to 38 days and the adults eclose in 10 to 12 days.

Generally, peak male eclosion occurs 2 to 3 days before peak female eclosion. Females have well-developed wings, but in North America they do not fly; by contrast, flight is common in Eurasian gypsy moth females (Leonard 1981). Mating usually occurs on the day of eclosion, followed immediately by oviposition; 300 to 1,000 eggs are deposited in the setae-covered egg mass.

The univoltine characteristic of the gypsy moth is mediated primarily by the time period spent in the egg (8 to 9 months). In the laboratory, a generation is completed in 220 to 240 days by cold treatment of eggs after the 21-day embryonation period and rearing under a constant 25°C.

Laboratory Rearing History

Strain Establishment

The NJSS, now in its 36th laboratory generation, was colonized at the APHIS-OMDC from eggs collected in the winter, probably November or December 1967, from a forest area near Blairstown, New Jersey (ODell et al. 1984). The gypsy moth population was building and had large egg masses. The number of eggs collected is unknown.

The wild eggs were stored in plastic containers at about 3°C (Appendix A, Ref. 5). Between February and September 1968, eggs were removed from refrigeration periodically and used to produce 7,516 NJSS-APHIS-F_1 egg masses (J. Tanner, USDA, APHIS-OMDC, pers. commun.). Recorded comments suggest that survival of F_1 was relatively poor, i.e., hatch was approximately 40 percent, primarily due to poor embryonation, and survival from hatch to adult was as low as 33 percent, e.g., from a sample of 1,227 neonates, only 405 adults were produced (Appendix A, Ref. 14). There were no records indicating cause of poor embryonation or high mortality. However, "wilt" or nuclear polyhedrosis virus (NPV), a natural disease responsible for causing gypsy moth populations to crash following outbreaks, was a serious problem in establishing and maintaining gypsy moth in the laboratory (Appendix A, Ref. 5; Bell et al. 1981). Records of experimental treatments to reduce the incidence of wilt

indicate that this problem was prevalent during the early laboratory generations of NJSS-APHIS.

At the time of establishment of NJSS-APHIS in the laboratory, artificial diets for rearing gypsy moth year round were just being developed (Appendix A, Ref. 5). Kennedy and Stevens (Appendix A, Ref. 5) indicate that NJSS-APHIS was initially reared on a modification of Vanderzant's (1966) basic wheat-germ diet. These modifications included an increase in formalin and the addition of ground hemlock needles, both for suppressing NPV. Between 1968 and the present, many other changes were made in gypsy moth diet to enhance survival, increase fecundity, and reduce costs. However, wheat germ has been the basic ingredient, and perhaps the most stable part of the rearing process.

Original Rearing Protocols

Prior to the development of mass-rearing techniques, about 1975 and NJSS-APHIS F_{13}, rearing protocols for maintaining the production colony simulated as closely as possible natural conditions and/or scientific recommendations. The following technique was used for generations F_1-F_{13} (Appendix A, Ref. 5).

Egg Incubation. Eggs were dehaired and combined in plastic mesh pouches for soaking (10 minutes) in 5.25-percent sodium hyperchlorite (NaOCl). After rinsing, eggs were air dried and treated with a solution of captan (ICI Americas, Inc., Goldsboro, NC; 100 g of 2.5 percent in 100 ml H_2O).[1] Eggs were incubated, 2,000 per plastic petri dish at 26.7°C and ≥80 percent relative humidity (RH). Eggs hatched in 3 to 7 days.

Larval Development. Neonates were placed in plastic petri dishes, approximately 15 per dish, with three cubes of artificial diet, and maintained at 25.6°C. After 10 to 12 days, the majority were second instars and transferred to 473.2-ml "food containers" with clear plastic lids. A 29.6-ml cup of diet was placed in each container, and a cup with fresh diet was added each week. Larvae were then reared in walk-in environmental chambers at 25° to 26°C, 65 to 75 percent RH, and a 14:10 light:dark period. Containers were inspected periodically; those containing mold and/or diseased larvae were discarded.

Pupal Development and Harvest. Males initiated pupation about 35 days after hatch, females about 4 to 5 days later. Pupae were harvested every 2 to 3 days beginning approximately 39 days after pupation and continuing for 2 weeks. After 2 weeks, 80 percent of those that would pupate had done so. The remaining 20 percent, representing small males and females, were discarded. From each pupal harvest 5 to 10 percent of the "superior" (largest and most lively) male and female pupae were chosen to maintain an adequate and healthy colony (ODell et al. 1984).

[1] Mention of a commercial or proprietary product does not constitute an official endorsement or approval by the U.S. Department of Agriculture or the Forest Service.

Eclosion, Mating, and Oviposition. Male and female pupae were paired according to predicted eclosion date and placed in #3 white Kraft paper bags for eclosion, mating, and oviposition. These processes occurred at about 24°C and 60 percent RH. Oviposition was complete in approximately 7 days; each bag with egg masses was then transferred to an embryonation chamber.

Embryonation and Diapause. Eggs were embryonated at 12.8°C (night) and 23.9°C (day) for approximately 20 days. The intact egg masses were removed by cutting a section from the bag and stored for 10 to 15 days at 12.8°C (ambient humidity). Eggs were then transferred to refrigerators set at 4.4°C (or 2.4°C) and stored there in plastic containers covered with a double layer of polyethylene, which allowed gas exchange but inhibited drying. Eggs were removed from refrigeration after 150 days (optimum for hatch; see review by Giese and Cittadino 1977).

Early History

After the initial spread of wild egg hatch over 6 months, subsequent generations generally followed the production schedule described. The number of production periods within a generation and the number of egg masses produced specifically for colony apparently varied (see Pupal Development and Harvest).

Although the basic rearing procedures described were sustained through the first 13 generations, mechanical failure of refrigerators containing NJSS-APHIS diapausing eggs caused significant loss of egg masses (J. Tanner, pers. commun.). Generations F_2 and F_3 were maintained at approximately the same level as F_1, that is, over the production period, about 7,500 egg masses per generation were produced. In 1971, most colony egg masses were lost due to a refrigerator breakdown. The absence of records for F_4 and only 40 egg masses recorded for F_5 suggest the majority of F_4 eggs did not survive. Records indicate that 102 F_6 egg masses were produced but that only 46 F_9 egg masses were available, suggesting another mechanical failure or some other mortality factor that reduced F_7 and/or F_8.

Production of NJSS-APHIS increased in generation F_{10}, i.e., F_{10} = 500 egg masses, F_{11} = 2,100 egg masses, F_{12} = 8,000 egg masses. Eighty F_{11} colony egg masses were used to produce 8,000 egg masses. Beginning with F_{10}, about 50 to 200 egg masses per week were used for maintaining the colony. Colony size was based on predicted needs for each production period (subcolony) in the next generation (J. Tanner, pers. commun.).

Changes in the APHIS-OMDC Rearing Protocol

In 1975, a USDA interagency research team began investigating methods for mass rearing gypsy moth (ODell et al. 1984). The goal, to develop a cost-effective production process capable of producing 25,000 to 50,000 insects a day, resulted in significant changes in diet composition and processing, rearing containers, mating protocols, and egg

storage environment (see Bell et al. 1981). Changes in colony production protocols followed those of mass production. APHIS-OMDC reports indicate that these changes were initiated in NJSS-$F_{13/14}$ (Appendix A, Refs. 2, 3). However, records for when modifications were made within each generation are incomplete. The major changes made between F_{13} and F_{20} are listed in Appendix B.

In 1985, Tanner and Baker (Appendix A, Ref. 17) reported that "straggling" or "stunting" was a major problem in the rearing program, with reduction in pupal yields of F_{28}, "at times" exceeding 40 percent. They characterized straggling as the "lack of growth" in newly hatched larvae, which also has been observed in wild strains in the laboratory (see ODell 1993 for discussion and analysis of straggling). Straggling was first documented as affecting certain subcolonies in F_{20} (Appendix A, Ref. 27). At that time, only larvae that had molted to the second instar within 11 days after hatch were used for colony production.

In 1985, a selection method was developed that might eliminate straggling (Appendix A, Ref. 17). Egg samples were removed from 100 egg masses 21 days before scheduled colony production. Eggs from each mass were placed on diet. After 11 days, the mean larval stage (MLS) was determined for each sample. The 18 egg masses with the highest MLS per sample were incubated 21 days later after 180 days of chill. Approximately 240 neonates per mass, 8 neonates per cup, were reared to pupation. Pupae were mixed in mating containers to potentially produce up to 75 different mating combinations. While this technique with minor modifications maintained production for several generations, it did not eliminate straggling.

Forest Service Rearing Methods

In 1980-81, NJSS-$F_{20, 21, 22}$ egg masses from the APHIS-OMDC were used to establish a stock colony at the USDA Forest Service's Insect Rearing Facility at Hamden, Conn. (C. ODell, USDA Forest Service, pers. commun.). Egg masses were shipped to the Hamden laboratory sporadically during 1981 and 1982. The number of NJSS-APHIS egg masses used weekly for producing the first Forest Service NJSS colony (NJSS-FS F_{23}) ranged from 8 to 31. In generation F_{24} the number of NJSS-FS F_{23} egg masses used for each subcolony ranged from 6 to 24. In subsequent generations (F_{25}-F_{31}), 15 egg masses were used to set up each subcolony and the rearing process given in Appendix C was established (C. ODell, pers. commun; ODell et al. 1985; Moore et al. 1985).

Changes in FS-CFHR Rearing Protocol

Prior to F_{32} (March 1988), 16 egg masses were incubated to ensure that 15 egg masses with good hatch were available for colony production. In F_{32}, the number of egg masses required increased sharply to 25, and in F_{33} it took 32 egg masses to ensure that 15 had good hatch within the 3-day incubation period. While some of this increase was due to variability in days to hatch, the major cause appears to be an increase in number of egg masses with poor hatch.

At the end of F_{32}, straggling was affecting colony production, reducing pupal subcolony egg-mass production by as much as 50 percent. A selection technique was initiated to ensure colony production. Fifty neonates from 20 egg masses (of 32 incubated) with the best hatch were transferred to a 15- x 100-mm plastic petri dish containing three cubes of diet. After 5 days at standard rearing conditions, 15 families were selected and the largest second-instar larvae were transferred to standard rearing containers for colony production. This technique appeared to increase the number of females produced and, thus, was changed in F_{33} to include all second instars.

Two changes were made in F_{33} to increase the number of families used in colony production. The number of egg masses incubated was increased to 45; after families with obvious straggling and/or dead or sick neonates are eliminated, the number of useable egg masses per subcolony ranged from 30 to 45. The number of mating pairs also was increased; 25 male and 25 female pupae are placed in 3.8-liter containers lined with butcher paper.

Possible Genetic Changes with Laboratory Rearing

Through the 36 generations in the laboratory, the NJSS has been subjected to many human-imposed changes intended to increase production efficiency and reduce costs. These changes have resulted in artificial selection, either intentional or unintentional, on several traits. In addition, many other genetic forces that result in genetic change have been operating, for example, the founder effect, random drift, Wahland's effect, and inbreeding and natural selection. In this section we speculate on the possible genetic changes that may have occurred over time in the NJSS; in the following section we present evidence for genetic change and discuss possible causes.

The source population for the NJSS probably included many different genotypes in varying frequencies, since it was a building population with large egg masses. Unknown numbers of egg masses were taken from this population to initiate the colony. When a sample of a population is used to establish a new colony, the selection always results in less genetic variation within the sample than was present in the population and frequently excludes and occasionally overrepresents rarer genotypes (Bartlett 1985). The fewer individuals that are used to establish the population and/or the poorer the sample in space or time, the greater the probability that, after a few generations, allelic frequencies in the new colony will differ greatly from those in the population of origin. These genetic changes result from random genetic drift following the establishment of the new colony and are called founder effects.

Thus, the NJSS would have started with a lower original genetic variability than the feral population from which it was collected and the differences between the NJSS and the original population would have been further accentuated with rearing due to random genetic drift. The intensity of the founder effects on the NJSS was further increased when samples of the original sample were used to start distinct

weekly subcolonies. So with rearing, the allelic frequencies in the NJSS as a whole would differ from those of the feral population from which it was started and also between individual subcolonies.

In generations F_1-F_{11}, the strain was maintained at low levels (≤ 50 EM per week) and nearly was lost twice. During this period, natural selection for genotypes adapted to the laboratory rearing regime would have had a strong influence and, with the added artificial selection for the largest (heaviest pupae), most "vigorous" 5 to 10 percent of the individuals at the pupal stage, only 5 percent of the colony was carried on each generation.

This process ultimately would result in intense inbreeding of the best adapted, largest, and most vigorous individuals. Based on this scenario, by generation F^{15} when the strain officially became the standard strain, genetic variability would have been greatly reduced and pupal weight should have increased. Rossiter (1987) showed that pupal weight was directly correlated with fecundity ($r = +0.81$), so as pupal weight increased with selection, fecundity also should have increased.

In the first 14 generations, sodium hypochlorite and captan were used to treat the eggs before hatch to reduce fungal, bacterial, and viral infection. These treatments were not effective at reducing NPV infection (Appendix A, Ref. 15), and as a result, several episodes of viral disease were responsible for reducing the number of individuals in the colony and potentially selecting for virus resistance. Since generation F_{15}, 10-percent formalin treatment of the eggs has been used and selection for virus resistance would have ceased except for removal of cups with dead or diseased larvae from the colony. The NJSS still may be more tolerant than feral populations to the NPV if the resistant individuals which were selected for in early generations are as fit as or more fit than susceptible individuals in the absence of the virus.

From the F_1 generation to present, artificial selection has been carried out for faster development to the pupal stage both intentionally and unintentionally. In the beginning generations, all but the slowest 20 percent of the pupae were harvested. The pupae that were not harvested probably were individuals that would have had extranumery instars (> 6 instars, females; > 5 instars, males). Beginning in generation F_{16}, pupae were harvested only once, on day 35. At day 35, even 6-instar females may not have had enough time to pupate and would then be excluded from the colony. Thus, in the process of selecting for faster pupation and in reducing rearing costs, not only should the NJSS be a faster developing strain but females with 6 instars may have been selectively removed from the population. The selection for families with second instars on the fourth day after receiving diet has been used since the middle of the F_{32} generation at the FS-BCID, and selection of families based on MLS at 11 days was used at the APHIS-OMDC for $F_{28\text{-}34}$. This may have further intensified the selection for fast developmental time.

In the first few generations, environmental conditions during prediapause and diapause were kept as closely as possible to those experienced by feral populations. This was a time-consuming, labor-intensive method that did not lend itself well to mass production. Consequently, the fluctuating temperatures and acclimation period were eliminated. Subsequently, when different chill periods and chill temperatures were evaluated, it was found that holding eggs longer in chill (150 to 180 days and at 8°C rather than 5°C) resulted in a shortened incubation time before hatch and in a greater percentage of the hatch occurring on the peak day of hatch. In the F_{22} generation, a 180-day chill at 8°C became the standard. These deviations from feral population conditions may have resulted in natural selection for insects that have different diapause requirements, and the consistency of conditions may have resulted in a reduction in the variation for these traits due to selection for a specific genotype that is best fit under these specific conditions. This reduced variability may be manifested in a more synchronous hatch and a different phenology of hatch compared with that for feral populations.

Rearing methods used in mating may have altered response to pheromones, flight behavior, and mating propensity. Originally, single pair matings were carried out in small white paper bags (#3 Kraft) which would restrict flight and reduce competition for mates. Later, group matings, 25 pairs in 3.8-liter paper containers, became the standard. These containers still restricted flight but now allowed competition for mates and the environment became more saturated with pheromone. The restricted flight may have selected for males that were less likely to fly, and the environment saturated with pheromone may have resulted in selection for altered pheromone release and response traits in the NJSS.

The completely artificial environment and closed population (no immigration) conditions under which the NJSS has been reared for several generations may have resulted in it being different from feral populations. If the NJSS is sufficiently different from feral populations, it may not be able to compete in the field. And since the only sources of new variation available to the NJSS are recombination and mutation, it may not be able to adapt to adverse conditions, i.e., anything that differs from standard rearing conditions (Mackauer 1972).

Evidence for Genetic Change or Stasis with Laboratory Rearing

Colony Data over Generations

Colony data from the APHIS-OMDC is available in quarterly or annual laboratory reports from 1976 to 1989 (see Appendix A for pertinent reports). The number of individuals used in samples and rearing techniques has changed over time but these data can be used to follow various traits over time to assess the influence of various rearing changes and selection. In the F_{15} generation on high wheat-germ diet (Appendix A, Ref. 3), percent hatch was 59 ± 8.9; at the next record, F_{20}, the hatch was 92.0 ± 5.4 percent (Appendix A, Ref. 27) and has remained above 80 percent since. During

this period, from F_{15} to F_{20}, rearing changed to a no-larval-transfer method and eggs were chilled for 150 days. It is not clear whether the increase in percent hatch can be attributed to the rearing changes, genetic changes (adaptation to the laboratory), or a combination of the two.

Data on fecundity from F_{13} to present are available in the reports listed in Appendix A and in Figure 1. Fecudity was below 900 eggs per female until F_{16} when the high wheat-germ diet became the standard and pupae were harvested only on day 35. Until the last three generations, fecundity has remained high and consistent. The recent drop in fecundity may be associated with straggling. In view of the changes in fecundity, changes in pupal weight of females would be expected. Figure 2 shows that female pupal weight (derived from the reports in Appendix A) has varied from generation to generation, following a pattern similar to that for fecundity. Male pupal weights have remained relatively unchanged over the 22 generations (Fig. 2).

The early increases in female pupal weights are consistent with the selection for the largest pupae, and the subsequent relative stasis occurs after selection was discontinued. The differences between the two diet types and methods (Figs. 1-2) are significant at P = 0.05. Thus, part of the observed increases in fecundity and female pupal weights can be attributed to changes in rearing methods.

Bell et al. (Appendix A, Ref. 3) described a particularly common pupal malformation, the "sunken thorax syndrome," as the incomplete sclerotization and excessive water loss in the thoracic region. They indicated its cause was due to age of diet (including age of wheat germ), and insect crowding as influenced by type of container and number of insects in each container. Pupal deformity was first reported during F_{14} and has been reported consistently since that time; often 80 to 90 percent of the female pupae have some evidence of the sunken thorax syndrome. Bell et al. (Appendix A, Ref. 3) indicated that larvae generally developed normally and pupated on time, and that pupal weights were normal to excessive. Pupal deformity generally did not affect adult eclosion, mating, or oviposition.

Mean days to pupation and to adult have decreased over time in the NJSS. Mean day to 50 percent pupation (± standard error) were 31.6 (± 2.1), 29.1 (± 0.9), and 26.6 (± 0.05) for males in generations 15, 20, and 30, respectively; the corresponding figures for females were 35.8 (± 2.7), 31.2 (± 1.2), and 25.5 (± 0.08) (Appendix A, Refs. 1, 18, and 30). The number of days required to complete larval development has decreased by 9 to 10 for females and 5 to 6 for males.

The more pronounced change in the female may indicate a change in the relative frequencies of instar types present in the colony. The change to 35-day pupal harvest, which

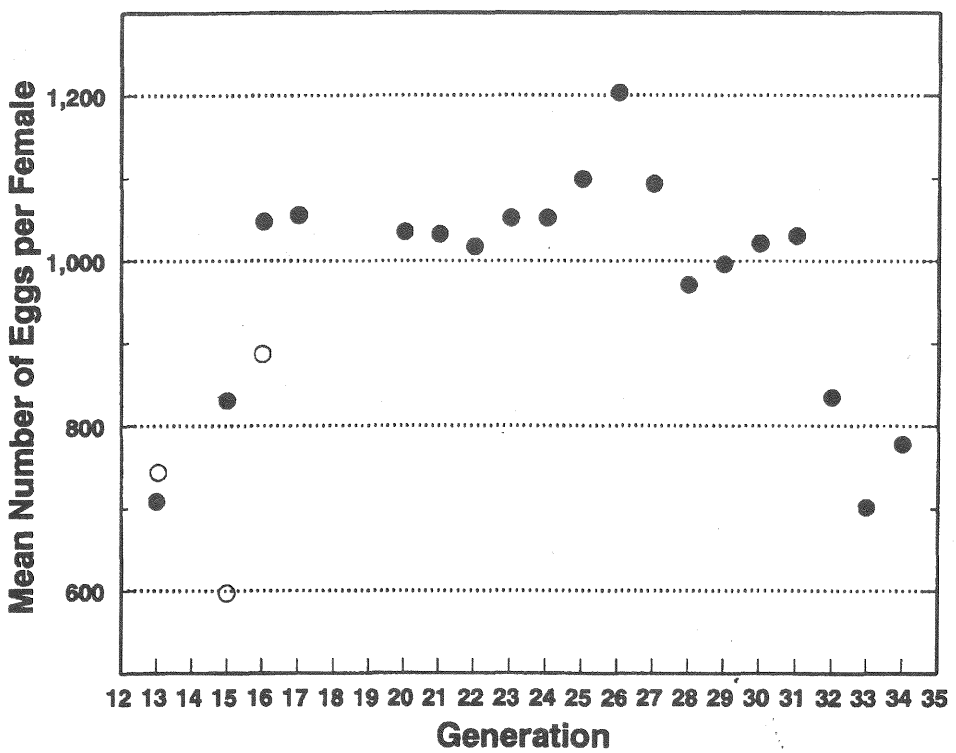

Figure 1.—Mean fecundity of NJSS-APHIS females from generation F_{13} to F_{34}. Means for individuals reared on high wheat-germ diet (solid) and modified tobacco hornworm diet (open) are given where available.

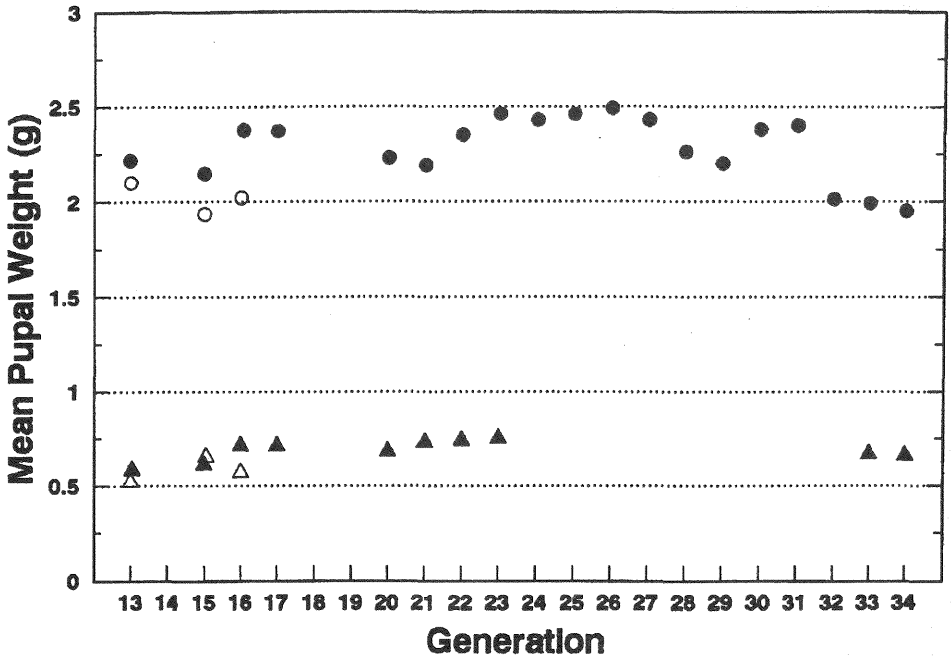

Figure 2.—Mean pupal weights of male (triangles) and female (circles) NJSS-APHIS from generations F_{13}-F_{34} (where available). Means for individuals reared on high wheat-germ diet (solid) and modified tobacco hornworm diet (open) are given where available.

occurred in F_{16}, probably has been responsible for the selection for a shorter developmental time to pupation.

Mean days to adult shows a decrease similar to time to pupation; however, data for F_{16} show that the changes made in diet initially resulted in a significant increase (Appendix A, Ref. 21). With a diet change from a high wheat-germ diet to modified hornworm diet at the fourth instar, mean days to adult for males was 40.5 (±1.0) and 40.8 (±1.0) for females. When high wheat-germ diet was used with no diet change, males took an average of 44.5 (± 0.7) days and females 43.9 (± 0.8) days.

Data on percent hatch and percent embryonation of monthly trials using egg-mass plugs are available for the NJSS colony reared by the Forest Service from F_{24} to F_{32} (Figs. 3-4). Percent hatch averaged 77.5 (±11.1) over this period with no trends apparent. Percent embryonation averaged 96.8 (± 2.9) with no significant changes. This would indicate that either there is no selection pressure being applied to these traits or that there is little phenotypic variability for these traits in the NJSS-FS.

The mean percentage of egg masses recovered, however, has increased in the NJSS-FS from generation F_{24} to present (Fig. 5). This increase may represent an adaptation to the mating containers, for example, increased mating propensity, altered pheromone release, altered pheromone response, or females becoming less sensitive to disruptions. A general increase in vigor does not account for this because egg-mass quality did not change as measured by percent hatch and percent embryonation.

The susceptibility of the NJSS-FS to NPV has decreased through the generations. From generations F_{14} to F_{24}, the NJSS-FS became more tolerant to NPV, as evidenced by a significant change in LC_{50} values ($P = 0.012$) (Briese and Podgwaite 1985). This decrease in susceptibility may be the result of early unintentional selection for individuals that were able to survive the early NPV epidemics before formalin was used. The continued decrease in susceptibility may be due to random genetic drift or higher fitness of the more tolerant individuals in the laboratory environment. The susceptibility of the NJSS-FS to *Bacillus thuringiensis* (Bt) has increased in the last few generations. For generations F_{24}-F_{30}, the response of the NJSS-FS to the standard Bt strain (HD-1-S-980) varied but did not differ significantly in slope or LC_{50} from generation to generation except for F_{25} (Dubois et al. 1989).

Similar variation in LC_{50} values were observed with other Bt strains and formulations (Dubois et al. 1989). However, in the last four generations, F_{32}-F_{35}, there has been a significant increase in susceptibility (decrease in LC_{50}) while the slope has remained constant (N. Dubois, USDA Forest Service, pers. commun.). During this period, there has been no change in the potency (International Units per mg) of the standard Bt preparation used (HD-1-S-1980) (N. Dubois, pers. commun.), so the changes in susceptibility are due to changes in the response of the NJSS-FS. These changes in Bt susceptibility may be due to the problems associated with

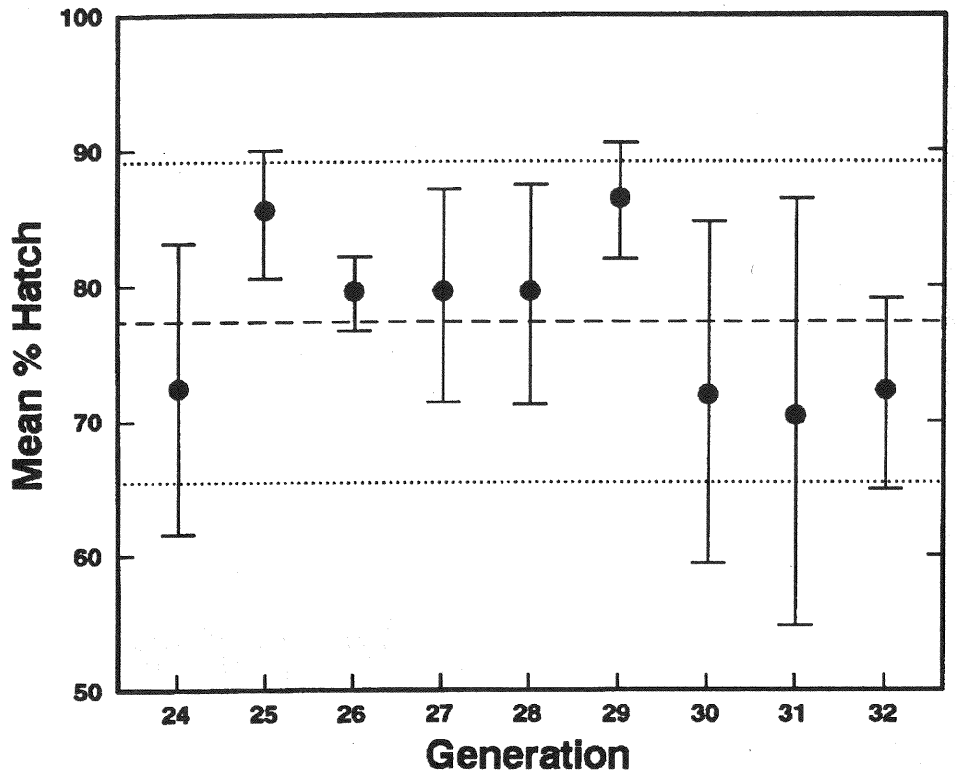

Figure 3.—Mean percent hatch of plugs taken monthly from NJSS-FS colony egg masses for generations F_{24}-F_{32}. Bars represent 95 percent CL; the mean over all generations is represented by dashed line and standard deviation of the overall mean by dotted lines.

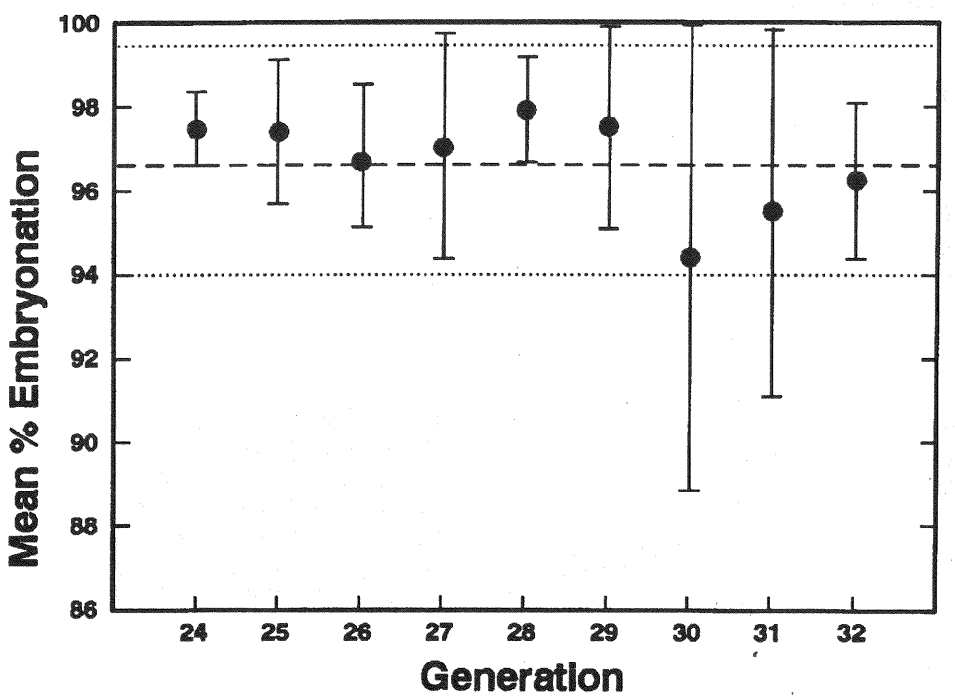

Figure 4.—Mean percent embryonation of egg-mass plugs used to determine percent hatch from NJSS-FS for generations F_{24}-F_{32}. Bars represent 95 percent CL; the mean over all generations is represented by dashed line and standard deviation of the overall mean by dotted lines.

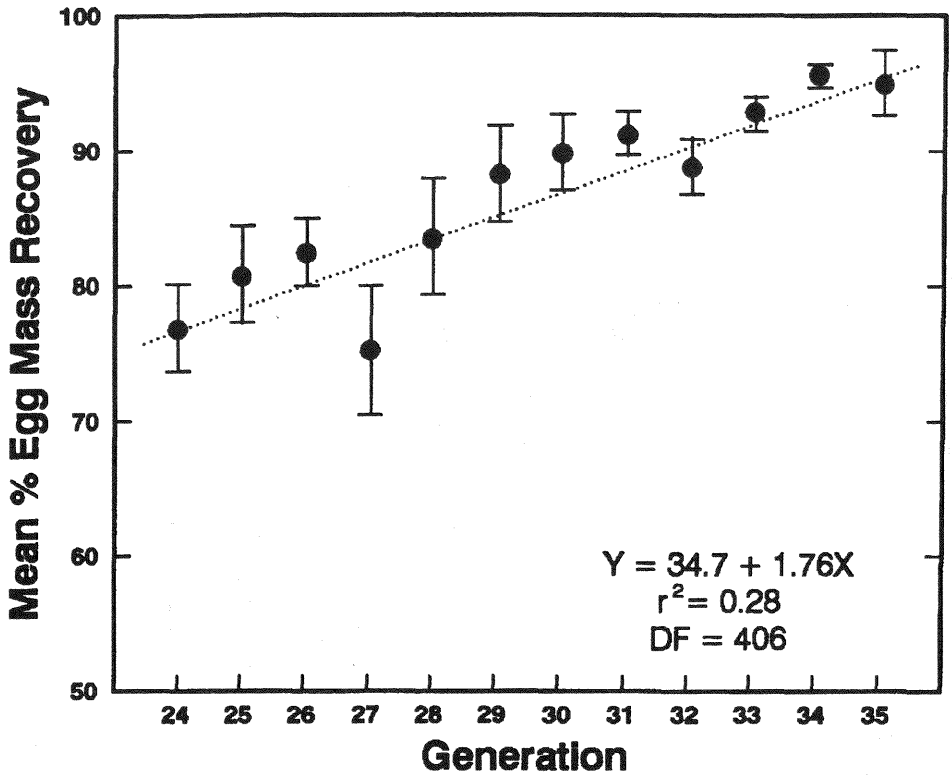

Figure 5.—Mean percent females laying egg masses in NJSS-FS colony production for generations F_{24}-F_{32}. Bars represent 95 percent CL and a regression line for these generations is represented by dotted line.

straggling, random genetic drift, or correlation of Bt susceptibility with traits selected for in the second-instar selection program, initiated in generation F_{32}.

Comparison of NJSS with Wild Strains in the Laboratory

F_{15} Generation Comparison

The F_{15} generation of the NJSS-APHIS was compared to a Pennsylvania wild strain using the high wheat-germ diet (Appendix A, Ref. 1, Table 1). Five egg masses were used and 16 to 20 individuals were reared per egg mass. There were few differences between the two strains except for developmental time. Time to pupation was 4 days longer for the wild strain. The NJSS F_{15} had slightly heavier pupae and slightly higher fecundity than the wild strain. This would indicate that despite the early selection for larger insects, little increase in pupal weight had occurred, but that early selection for faster developing strain (by stopping pupal harvest before all individuals had pupated) had resulted in significant decreases in time to pupation.

Electrophoretic Data

Allozyme variation at 20 loci was compared for the NJSS ($F_{20\text{-}21}$) and 14 wild strains (Harrison et al. 1983). At the phosphoglucomutase (Pgm) locus, the frequencies of the two alleles present in the 14 North American wild strains varied little. However, the h allele, labeled "h" because it was the 8th in anodal mobility with "a" being the greatest, averaged 0.02 percent in the wild strains and occurred at relatively high frequency (> 0.5) in the NJSS. Whether the high frequency of Pgm^h in the NJSS is the result of an initial founder effect, genetic drift in subsequent generations, or selection under laboratory rearing conditions is unknown.

Table1.—Comparison of performance of NJSS-APHIS F_{15} and a wild strain of gypsy moth collected in Pennsylvania (both reared on high wheat-germ diet)

Item	Sex	NJSS F_{15}	PA (wild)
Days to pupation	Male	31.6 (2.1)[a]	35.4 (2.0)
	Female	35.8 (2.7)	40.1 (3.3)
Days to adult	Male	43.8 (1.4)	48.1 (2.0)
	Female	46.2 (2.7)	50.2 (3.1)
Pupal weight (g)	Male	0.59 (0.06)	0.53 (0.06)
	Female	1.60 (0.30)	1.70 (0.41)
Fecundity		543 (120)	553 (70)

[a] Mean value with standard deviation in parentheses.

F_{33} Generation Comparison

During NJSS-FS generation F_{33}, egg masses from five areas: Wilton, Connecticut (CT), Oak Hill, Vermont (VT),

Beltsville, Maryland (MD), Leidy Township, Pennsylvania (PA), and Montreal, Canada (CANADA), were brought into the laboratory to be evaluated as possible backup strains. Egg masses were held at 5°C until the end of March when they were incubated to initiate hatch. Egg masses were packeted in 20-mesh monofilament saran screening (National Filter Media Corp., Hamden, CT 06514) and disinfected with 10 percent formalin. Egg masses were incubated and individuals reared at 25°C for 16 hours of light and 20°C for 8 hours of dark at 60 ± 10 percent RH. Each day for 5 days, beginning on the first day that ≥ 10 neonates hatched, 10 individuals were set up and reared individually from each of 20 egg masses per area. Ten NJSS-FS egg masses also were hatched in the same way. Each individual was provided 15 ml of high wheat-germ diet in a 59-ml clear plastic container with an opaque white lid. Each larva was observed daily to determine if it had molted or prepupated and the Julian date recorded on the cap if a change had occurred. Pupae were sexed, weighed, and pupal deformities assessed 24 h after pupation was observed. Sixteen pupae, either all males or all females, were held in 19.1- x 19.1- x 7.6-cm corrugated cardboard boxes, each in a 3.8-cm-square subdivision. The box was covered with clear plastic wrap on which each individual's six-digit number was recorded. Eclosion was recorded for each individual and random individual matings were carried out by placing one female and one male moth from a given area in a white paper bag (#5 Kraft).

Egg masses were harvested, each cut out individually, 10 days after mating. The parent's numbers and mating date were recorded on the paper next to the egg mass. All harvested egg masses were moved to 15°C for 13 days then to 5°C for 150 days. Seventy egg masses from each area and 39 from the NJSS-FS were hatched in the same environmental conditions used in the first generation. Hatch phenology, percent hatch of embryonated eggs, and fecundity were determined for each egg mass.

The developmental data for the five wild strains were more like each other than like the NJSS-FS, so only data from the CT wild strain are presented for comparison with the NJSS-FS.

Hatch and fecundity data for the parental generation could not be compared because of the different environmental conditions the egg masses had experienced; therefore, data for the progeny egg masses (F_1 generation) are presented. There was a statistically significant difference in fecundity between the NJSS-FS, 946 ± 159, and the CT strain, 685 ± 244. The mean percent hatch of embryonated eggs also differed between the two strains (NJSS-FS 91 ± 11 percent versus CT 76 ± 34 percent). Also, the variance for these two traits was significantly smaller for the NJSS than for the CT strain. From these data we conclude that the NJSS-FS has been selected, either through natural or artificial means, for greater fecundity and percent hatch under laboratory conditions. Increased fecundity and hatch is advantageous for mass production but the reduced variability may make the NJSS-FS less able to adapt to adverse conditions.

The hatch profile of the NJSS-FS was different from that of the CT wild strain. The NJSS-FS hatch began sooner, had a greater percent hatch on the peak day, and finished sooner than the CT strain (Fig. 6). The variation in time to initial hatch followed a similar pattern (Fig. 7). For both initiation of hatch and profile of hatch, the NJSS-FS was more synchronous and less variable than the CT strain, possibly the result of inbreeding in the NJSS and/or selection on this trait.

For 5-instar males and females and 6-instar males, the NJSS-FS developed to the pupal stage significantly ($P = 0.05$) faster than the CT strain (Table 2, Figs. 8-9). However, the difference between the means for the 6-instar males may not be biologically significant since the frequency distributions are similar (Fig. 9). For 6-instar females there was no significant difference between the two strains (Table 2, Fig. 8). There were insufficient numbers of 4-, 7-, and 8-instar individuals to make a statistical comparison. There were significant differences in the number of days to pupation for the various instar types and between the sexes within an instar type. If we compensate for the degree-day difference between this alternating temperature regime and the constant temperature regime used for colony production (approximately 2 days) and for the difference between rearing containers (approximately 1 day), we can determine what individuals are being selected for in the 35-day harvest method. For the conditions used in this experiment, 38 days would be the equivalent harvest time and would result in the exclusion of most of the 6-instar females and all of the 7- and 8-instar females (Fig. 8). Only 4- or 5- instar males would have been harvested and used.

The small numbers of 6-, 7-, and 8-instar individuals in the NJSS compared to the CT strain suggest that they have been selected against. Figure 10 shows the difference in the relative numbers of various instar types in the two populations. The 5-instar female has become the dominant type in the NJSS-FS while 6 instars predominate in the wild strain. Extranumery instars were all but missing in the NJSS-FS and a 4-instar male type also had appeared. This is consistent with the NJSS-APHIS colony (Appendix A, Ref. 23).

The within- and between-family variances for days to pupation in the NJSS-FS tended to be less than those of the CT strain (Table 2). Between-family variance components for the 6-instar females and the 5- and 6-instar males were much smaller for the NJSS-FS than for the CT strain. Also, for both male and female 5-instar types, the within-family variance for the NJSS-FS was much lower than that of the CT strain and was significantly lower than the between-family variance component (Table 2). The reduced variance and mean days to pupation in the NJSS-FS are evidence for inbreeding in the strain and/or selection for a faster developing strain. The founder effect and random genetic drift also may have contributed to the reduced variance.

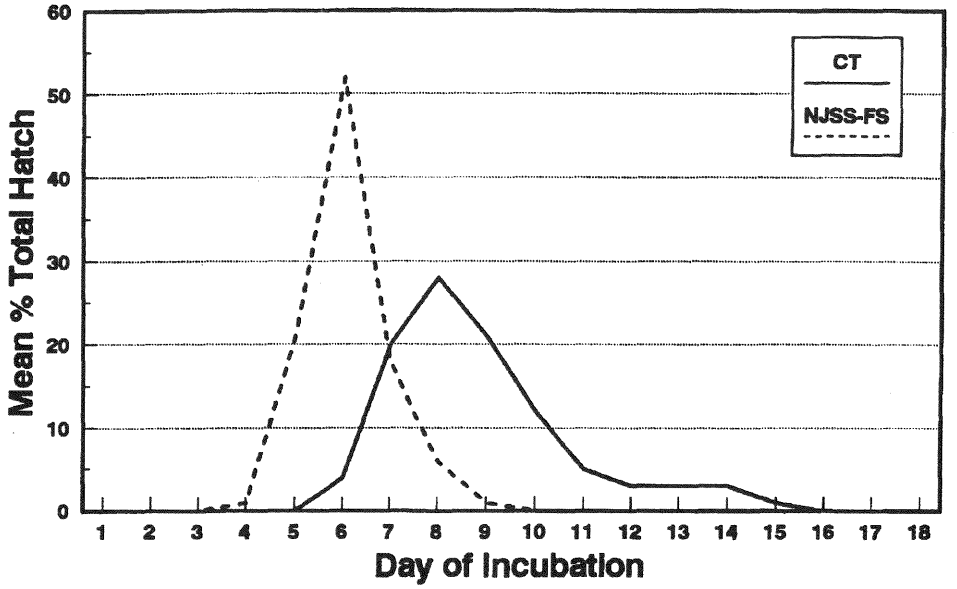

Figure 6.—Hatch phenology for *Lymantria dispar* egg masses held at 5°C for 150 days and incubated at 20°(8 hours) / 25°C (16 hours).

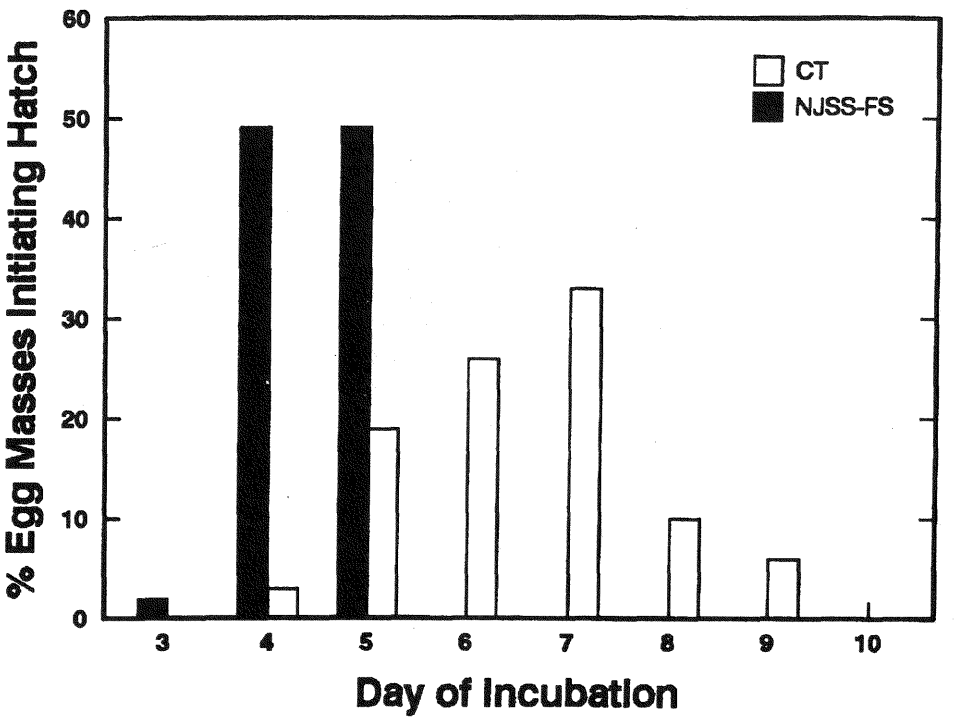

Figure 7.—Percent *Lymantria dispar* egg masses initiating hatch on each day of incubation at 20° (8 hours) / 25°C (16 hours). Egg masses were chilled for 150 days at 5°C.

Table 2.—Comparison of mean days to pupation grouped by sex and number of instars for NJSS-FS and CT strains[a]

Strain	Sex	No. of instars	n	Mean	Degrees of freedom		Estimated variance component[b]		P level
					Family	Individual	Family	Individual	
CT	Female	5	63	37.81	16	46	585.27	43.44	0.62
NJSS	Female	5	166	34.63	9	156	672.38	15.54	<0.01
CT	Female	6	214	45.86	19	194	2182.79	51.81	0.03
NJSS	Female	6	47	44.38	8	38	816.22	70.54	0.20
CT	Female	7	29	65.55	14	14	517.21	31.52	0.36
NJSS	Female	7	1	55.00	—	—	—	—	—
CT	Female	8	10	71.00	—	—	—	—	—
CT	Male	5	314	36.33	19	294	1152.37	30.82	0.01
NJSS	Male	5	208	32.68	9	198	950.35	15.14	<0.01
CT	Male	6	42	56.79	17	23	716.14	38.29	0.39
NJSS	Male	6	12	51.67	5	6	151.71	63.05	0.71
CT	Male	7	12	64.50	—	—	—	—	—
NJSS	Male	7	1	56.00	—	—	—	—	—

[a] Reared individually at 25°C for 16 hours of light and 20°C for 8 hours of dark.
[b] F test compares family and individual variance to determine whether there is a significant family-variance component.

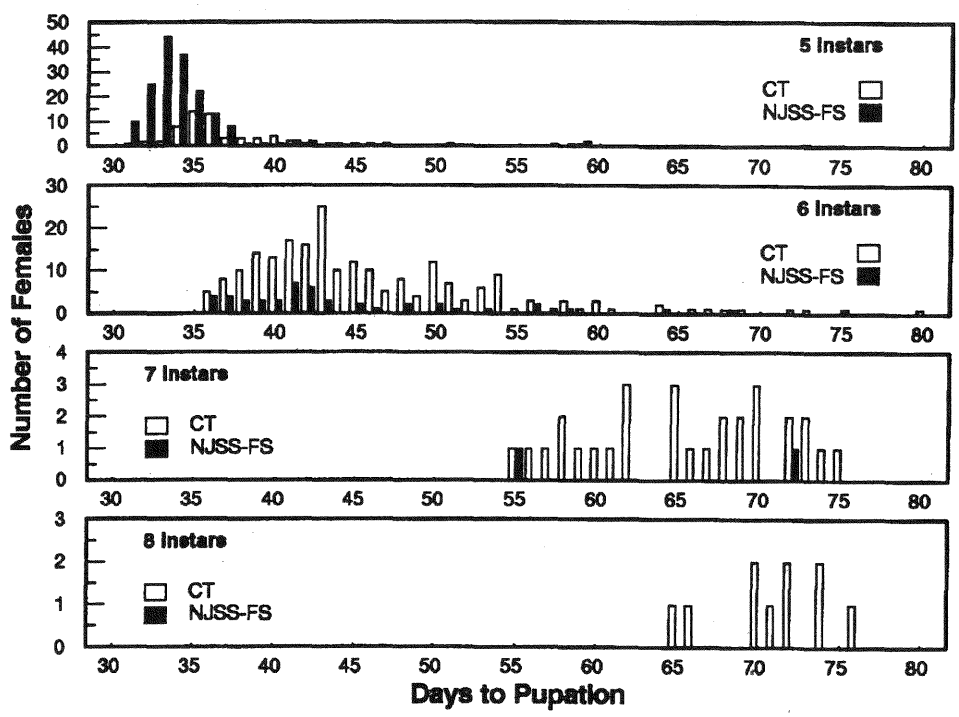

Figure 8.—Comparison of days to pupation at 20° (8 hours) / 25°C (16 hours) for individually reared *Lymantria dispar* females from two strains (CT and NJSS-FS) and four instar types (5 to 8).

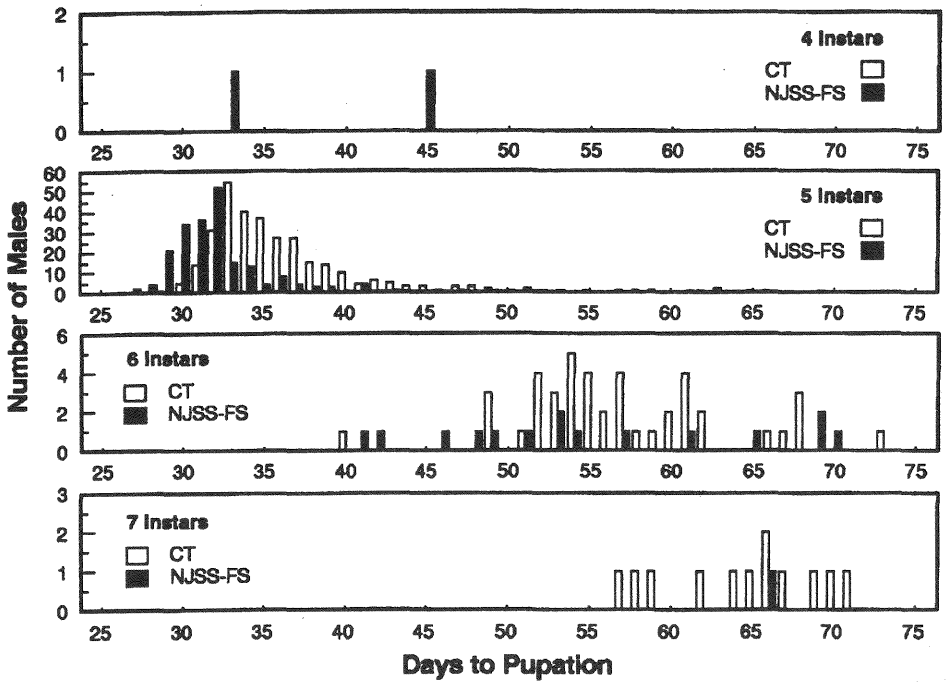

Figure 9.—Comparison of days to pupation at 20°(8 hours) / 25°C (16 hours) for individually reared *Lymantria dispar* males from two strains (CT and NJSS-FS) and four instar types (4 to 7).

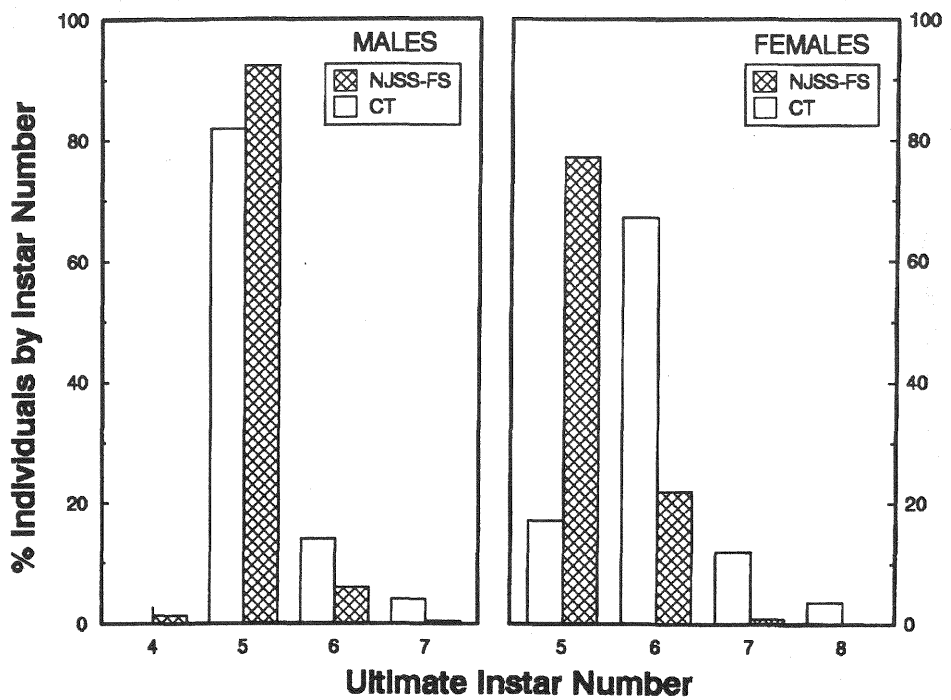

Figure 10.—Proportion of different instar types for *Lymantria dispar* males and females of two strains (CT and NJSS-FS) individually reared at 20°(8 hours) / 25°C (16 hours).

For all instar types where a comparison was possible, the NJSS-FS had significantly (P = 0.05) heavier pupal weights for both males and females compared with the CT strain (Table 3, Figs. 11-12). In addition, the between-family variance component for the NJSS-FS 5-instar males and females and the 6-instar females was much smaller than that of the CT strain while the within-family variance components were similar (Table 3). The heavier mean pupal weights and the reduced between-family variance suggest that selection for heavier pupae has occurred and that inbreeding in the NJSS also may have occurred. The similar within-family variance may indicate that inbreeding effects have not been extreme for this trait.

Bt and NPV Comparisons

The susceptibility to NPV and Bt of second instars from the third generation (F_2) of the wild strains was compared with that of the NJSS-FS generation F_{35}. Five replications of 10 individuals each were completed for six concentrations and a control. The concentrations for NPV were 10^6, 10^5, 10^4, 10^3, 10^2, and 0 polyhedral inclusion bodies per ml of diet. The concentrations of Bt were 12.5, 6.25, 4.69, 3.125, 1.56, and 0 µg of HD-1-S-1980 per ml of diet. Method of bioassay are given by Dubois (1986) for Bt and Rollinson and Lewis (1973) for NPV. Bioassays were conducted in 1990 by Normand Dubois (Bt) and John Podgwaite (NPV) at the Forest Service's Northeastern Forest Experiment Station Laboratory at Hamden, Connecticut. Probit regression lines were estimated with the probit option of POLO-PC (LeOra Software 1987) and compared with a likelihood ration test for equality (Russell et al. 1977).

The concentration-mortality lines for NPV had significantly different intercepts and the NJSS had the highest LC_{50} value (Table 4). The concentration-mortality lines for Bt were not significantly different at the 0.05 level and the NJSS-FS had the lowest LC_{50} value (Table 5). From these results we conclude that the NJSS-FS is more tolerant of NPV but is not different from the wild strains in response to Bt. However, Rossiter et al. (1990) found that the NJSS-APHIS strain (F_{31}) had an LC_{50} approximately twice that of three wild Pennsylvania strains that were tested. These differences in Bt susceptibility between the two NJSS colonies may be due to random genetic drift, founder effects, or the associated straggling problem, while the differences in NPV susceptibility may be the result of selection as previously discussed.

Response to Secondary Compounds

Feeding response experiments in which secondary plant compounds were incorporated into artificial diet suggested that the NJSS may be less susceptible to some of these compounds than wild larvae. These include phenolic glycosides extracted from aspen leaves (Lindroth and Hemming 1990; Lindroth and Weisbrod 1991) and catalpside, an iridoid glycoside (Bowers and Puttick 1989). However, when tannic acid was incorporated into artificial diet the NJSS had a similar response to that of wild strains in two of four trials (M. Montgomery, USDA Forest Service, pers. commun.). This would indicate that adaptation to the laboratory, inbreeding, and artificial selection (i.e., for speed of development or weight) may have altered the response of the NJSS to some secondary compounds found in leaves.

Table 3.—Comparison of pupal weight grouped by sex and number of instars for NJSS- FS and CT strains[a]

Strain	Sex	No. of instars	n	Mean	Degrees of freedom		Estimated variance component[c]		P level
					Family[b]	Individual	Family	Individual	
CT	Female	5	63	1.90	16	46	2.757	0.086	0.03
NJSS	Female	5	166	2.22	9	156	0.956	0.042	0.01
CT	Female	6	214	1.91	19	194	6.725	0.158	<0.01
NJSS	Female	6	47	2.33	8	38	1.165	0.131	0.36
CT	Female	7	29	1.47	14	14	1.656	0.204	0.81
NJSS	Female	7	1	2.28	—	—	—	—	—
CT	Female	8	10	1.77	—	—	—	—	—
CT	Male	5	314	0.72	19	294	1.179	0.130	<0.01
NJSS	Male	5	208	0.81	9	198	0.398	0.006	<0.01
CT	Male	6	42	0.45	17	23	0.200	0.014	0.60
NJSS	Male	6	12	0.68	5	6	0.292	0.019	0.10
CT	Male	7	12	0.56	—	—	—	—	—
NJSS	Male	7	1	0.32	—	—	—	—	—

[a] Reared individually at 25°C for 16 hours of light and 20°C for 8 hours of light.
[b] Family = egg mass.
[c] F test compares family and individual variance to determine whether there is significant family-variance component.

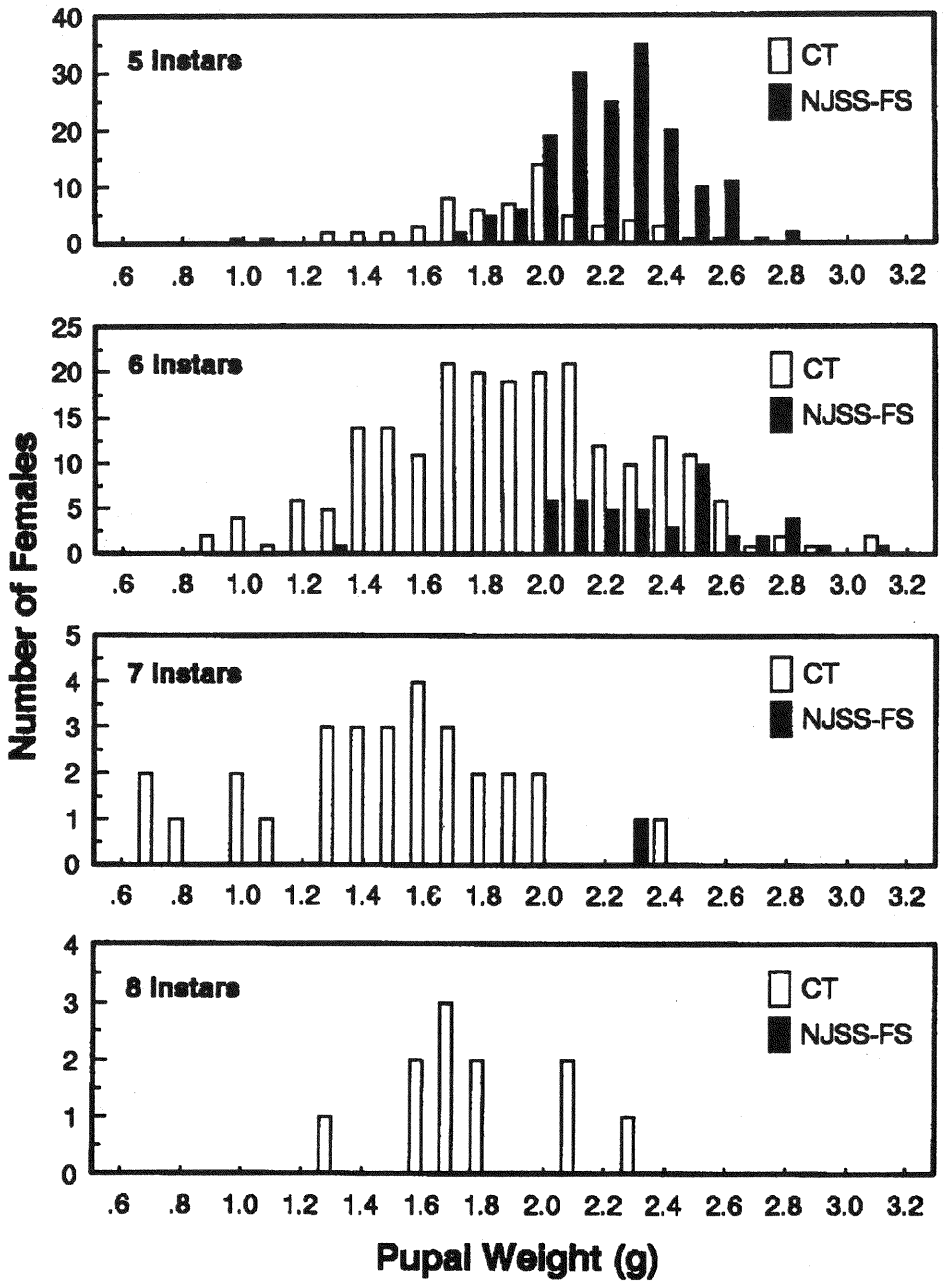

Figure 11.—Comparison of 24-hour pupal weights of individually reared *Lymantria dispar* females from two strains (CT and NJSS-FS) and four instar types (5 to 8).

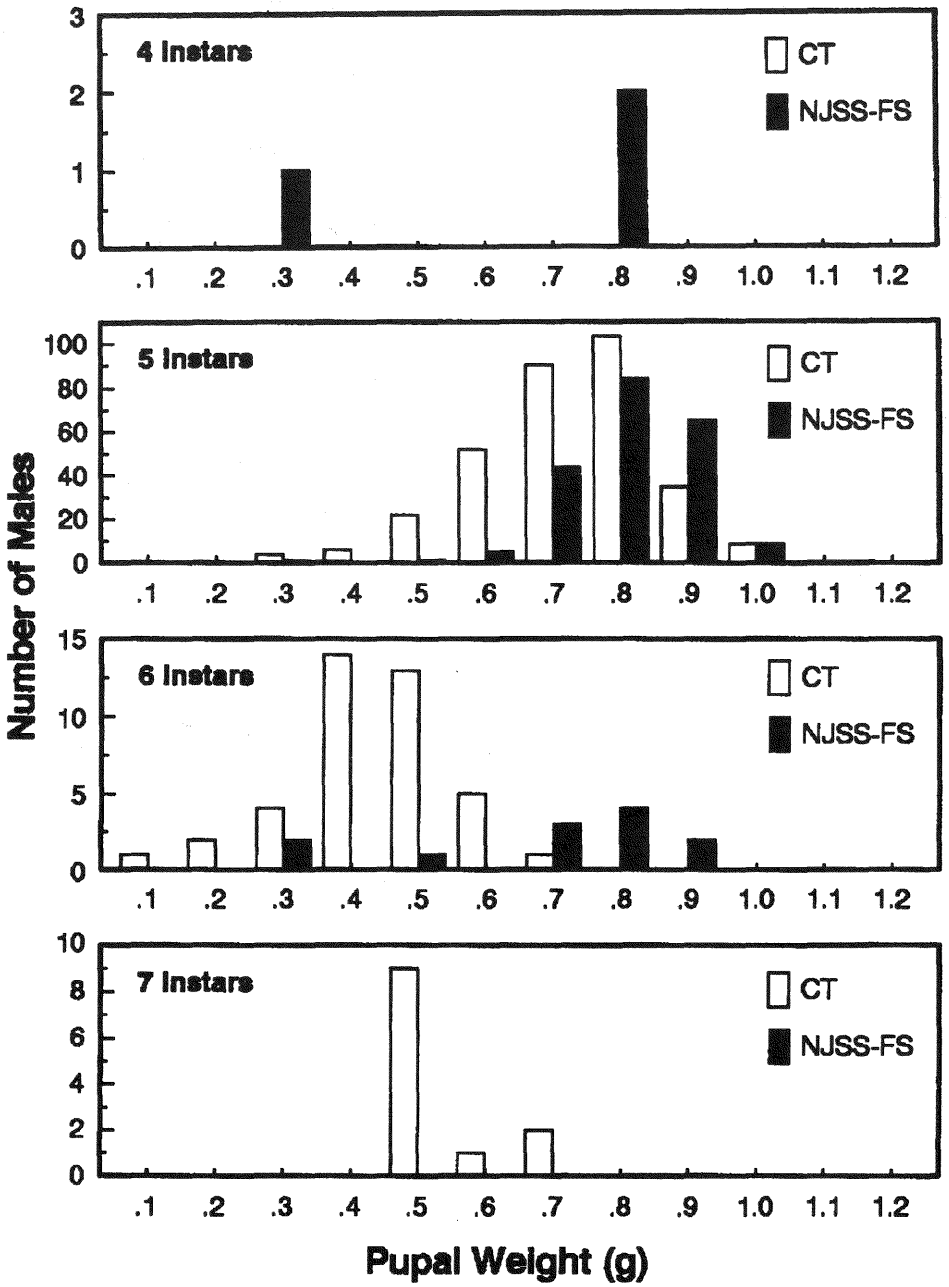

Figure 12.—Comparison of 24-hour pupal weights of individually reared *Lymantria dispar* males from two strains (CT and NJSS-FS) and four instar types (4 to 7).

Table 4.—Susceptibility of second instars from NJSS-FS and wild strains to Gypchek (Hamden secondary standard), a nuclear polyhedrosis virus

Strain	n	Slope ± SE	$LC_{50} \times 10^3$	(95 percent CL)[a]
CT	299	2.12 ± 0.31	8.84	(6.25-12.41)
VT	298	2.40 ± 0.40	5.55	(4.01- 7.65)
PA	298	2.36 ± 0.39	6.02	(4.35- 8.34)
CANADA	295	1.55 ± 0.21	3.70	(2.47- 5.50)
NJSS-FS	300	2.38 ± 0.35	12.52	(9.12-17.36)

[a] Polyhedral inclusion bodies per ml of diet.

Table 5.—Susceptibility of second instars from NJSS-FS and wild strains to *Bacillus thuringiensis* HD-1-S-1980 (16,000 IU/mg)

Strain	n	Slope ± SE	$LC_{50} \times 10^3$	(95 percent CL)[a]
CT	300	3.24 ± 0.38	4.46	(3.73-5.37)
VT	300	2.53 ± 0.33	5.05	(4.17-6.26)
MD	298	2.77 ± 0.35	5.32	(4.31-6.76)
PA	300	2.98 ± 0.36	5.18	(4.47-6.08)
CANADA	300	2.97 ± 0.36	4.96	(4.31-5.72)
NJSS-FS	299	3.43 ± 0.39	4.36	(3.65-5.20)

[a] µg HD-1-S-1980 per ml of diet.

Developmental and Behavioral Competitiveness Studies. Many comparisons of competitiveness between the NJSS and wild strains have been made in conjunction with the sterile male and inherited sterility gypsy moth programs (see Mastro et al. 1989 for review). Table 6 summarizes the results of several studies, both developmental and behavioral in nature, conducted between 1978 and 1989. There were significant differences between the NJSS and wild strains in fewer days to 50 percent hatch, more frequent dispersal of NJSS neonates, faster development of the NJSS larvae, and altered pheromone emission by adult NJSS females. The fewer days to 50 percent hatch in NJSS (when overwintered outside) compared to wild strains corresponds with laboratory observations (Fig. 6). Faster development of the NJSS larvae in the field corresponds with the results observed in the laboratory. Altered pheromone emission by females may be the result of adaptation to the laboratory mating regime. The ability to synchronize hatch in the field with that of the feral population by means of altering the diapause conditions and length suggests that the difference in hatch phenology seen in the laboratory under different conditions represents an adaptation to the laboratory, but that some phenotypic and/or genetic variability has been maintained.

Conclusion

Significant phenotypic changes in the NJSS have occurred over 35 generations in the laboratory. The change in phenotypic variability includes reduced asynchrony in hatch and a reduction in the occurrence of different instar types. Other phenotypic changes include increases in fecundity, percent hatch, pupal weight, pupal deformities, and egg-mass recovery. There also was a decrease in days to pupation and days to adult. Susceptibility to Bt and virus apparently has increased or decreased, respectively, with laboratory rearing.

Laboratory and field comparison of the NJSS with wild and near wild strains indicates significant differences. Genetic differences are evidenced by the reduced within- and between-family variance in pupal weight and days to pupation in the NJSS compared to a wild strain. In addition, several phenotypic differences appear to have occurred in the NJSS when compared to wild strains. NJSS develops faster, has fewer instar types, is more fecund, and is more synchronous in hatch; also, percent hatch of embryonated eggs is greater. NJSS also appears to be more resistant to virus, has a relatively high Pgm^h frequency in allozyme comparisons, and apprently is less responsive to secondary plant chemicals. In addition, female pheromone emission is greater. However, field studies of larval and adult behavior suggest that laboratory-reared NJSS males and females are competitive with their wild counterparts, indicating that although adaptation to the laboratory for development/life history traits has occurred, little behavioral adaptation of traits measured has taken place.

Phenotypic and genetic changes that have occurred in the NJSS could be the result of the founder effect, inbreeding, natural or artificial selection (which has been demonstrated), random genetic selection, or, more likely, a combination of these. Regardless of the mechanism effecting change, use of NJSS for research and development should be assessed carefully relative to the effects of domestication and the need to equate the performance of the NJSS to a specific research/development objective. Synchrony of development, increase in speed of development, and increased weight makes NJSS a good strain for NPV production when straggling is not a problem. These same development traits along with excellent adult male competitiveness have stimulated and enhanced research on the use of sterile males for management of low-density gypsy moth populations. NJSS larvae also are used extensively for both applied and basic research. The extensive differences in development/life history traits between the NJSS and wild strains suggest caution when using NJSS larvae in bioassays. The information provided in this paper should be used first in the selection of an experimental insect and then in the interpretation of results.

Table 6.—Comparison of development and behavior between NJSS-APHIS and wild strains

Method[a]	Trait	Generation	Results	Reference[b]
FC	Hatch synchrony	F_{20}	When egg masses were overwintered outside, mean days to 50 percent hatch in NJSS egg masses occurred significantly earlier than in wild egg masses	7
MRR	Neonate dispersal	F_{22}	NJSS dispersed more frequently than wild strains	6
FC	Diurnal and nocturnal activity patterns, all stadia	F_{33}	Similar patterns exhibited for NJSS and wild strains	4
FC	Large larval dispersal	F_{30}	Same patterns for NJSS and wild strains	13
FC	Development on black oak terminals	F_{23}	Survival, days to pupation, and pupal weights for both sexes not significantly different for NJSS and wild strain	9
		F_{30}	NJSS developed faster than a wild strain	M. Montgomery, pers. commun.
MRR	Pupal eclosion periodicity	F_{15}	No difference between NJSS and wild strains	10
MRR	Adult dispersal periodicity	F_{15}	No difference between NJSS and wild strains	10
LC	Adult male propensity to fly (actograph)	F_{20}	No difference between NJSS and wild strains	8
LC FC	Adult male periodicity of activity (actograph)	F_{20} F_{16}	No difference between NJSS and wild strains	8 11
FC	Horizontal and vertical distribution of adults in test canopy	F_{16} F_{33}	No difference between NJSS and wild strains	11 4
LC	Diel periodicity of pheromone emission by adult females	F_{22}	No difference between NJSS and wild strains	Charlton and Cardé 1982
LC	Mean pheromone emission rate by adult females	F_{22}	Longer for NJSS than wild females	Charlton and Cardé 1982
LC	Peak pheromone emission from 2-day-old adult females	F_{22}	Higher for NJSS than wild females	Charlton and Cardé 1982
MRR	Response of adult males to pheromone sources and periodicity of response	F_{16}	No difference between NJSS and wild strains	11
LC	Response of adult males to pheromone (flight tunnel)	$F_{16\text{-}17}$	No difference between NJSS and wild strains	Waldvogel et al. 1982
FC	Frequency of mating of males of various ages	F_{16}	No difference between NJSS and wild strains	10
FC	Periodicity of mating		No difference between NJSS and wild strains	Mastro et al. 1989
FC	Length of mating		No difference between NJSS and wild strains	Mastro et al. 1989
FC	Propensity of males to mate	F_{27}	No difference between NJSS and wild strains	ODell, unpublished data
FC	Oviposition of fertile egg mass by females once mated	F_{28}	No difference between NJSS and wild strains	12

[a] MRR = Field comparison using mark-release-recapture techniques; FC = confined field comparison; LC = laboratory comparison.
[b] Numbers refer to references in Appendix A.

Acknowledgments

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Literature Cited

Bartlett, A. C. 1984. **Genetic changes during insect domestication.** In: King, E. G.; Leppla, N. C., eds. Advances and challenges in insect rearing. ARS Publ. New Orleans, LA: U.S. Department of Agriculture, Agricultural Research Service: 2-8.

Bartlett, A. C. 1985. **Guidelines for genetic diversity in laboratory colony establishment and maintenance.** In: Singh, P.; Moore, R. F., eds. Handbook of insect rearing, vol. 1. Amsterdam: Elsevier Press: 7-17.

Bell, R. A.; Owens, D. C.; Shapiro, M.; Tardif, J. R. 1981. **Development of mass rearing technology.** In: Doane, C. C.; McManus, M. L., eds. The gypsy moth: research toward integrated pest management. Tech. Bull. 1584. Washington, DC: U.S. Department of Agriculture, Forest Service: 599-633.

Briese, D. T.; Podgwaite, J. D. 1985. **Development of viral resistance in insect populations.** In: Maramorsch, K.; Sherman, K. E., eds. Viral insecticides for biological control. New York: Academic Press: 361-398.

Bowers, M. D.; Puttick, G. M. 1989. **Iridoid glycosides and insect feeding preferences; gypsy moths (*Lymantria dispar*, Lymantriidae) and buckeyes (*Jyunonia coenia*, Nymphalidae).** Ecological Entomology. 14: 247-256.

Charlton, R. E.; Cardé, R. T. 1982. **Rate and diel periodicity of pheromone emission from female gypsy moths (*Lymantria dispar*) determined with a glass-adsorption collection system.** Journal of Insect Physiology. 28: 423-430.

Dubois, N. R. 1986. **Synergism between β-exotoxin and *Bacillus thuringiensis* subspecies *kurstaki* (HD-1) in gypsy moth, *Lymantria dispar*, larvae.** Journal of Invertebrate Pathology. 28: 146-151.

Dubois, N. R.; Huntley, P. J.; Newman, D. 1989. **Potency of *Bacillus thuringiensis* strains and formulations against gypsy moth and spruce budworm larvae: 1980-1986. Gen. Tech. Rep. NE-131. Broomall, PA: U.S. Department of Agriculture, Forest Service, Northeastern Forest Experiment Station. 25 p.**

Giese, R. L.; Cittadino, M. L. 1977. **Relationships of the gypsy moth to the physical environment. II. Diapause.** Staff Pap. Ser. No. 6. Madison, WI: University of Wisconsin: 1-13.

Harrison, R. G.; Wintermeyer, S. F.; ODell, T. M. 1983. **Patterns of genetic variation within and among gypsy moth, *Lymantria dispar* (Lepidoptera: Lymantriidae) populations.** Annals of the Entomological Society of America. 76: 652-656.

Joslyn, D. J. 1984. **Maintenance of genetic variability in reared insects.** In: King, E. G.; Leppla, N. C., eds. Advances and challenges in insect rearing. ARS Publ. New Orleans, LA: U.S. Department of Agriculture, Agricultural Research Service: 20-29.

Leonard, D. E. 1981. **Bioecology of gypsy moth.** In: Doane, C. C.; McManus, M. L., eds. The gypsy moth: research toward integrated pest management. Tech. Bull. 1584. Washington, DC: U.S. Department of Agriculture, Forest Service: 9-29.

LeOra Software, Inc. 1987. **POLO-PC: a user's guide to probit or logit analysis.** Berkeley, CA: LeOra Software, Inc.

Lindroth, R. L.; Hemming, J. D. C. 1990. **Responses of the gypsy moth (Lepidoptera: Lymantriidae) to Tremubicin, an aspen phenolic glycoside.** Environmental Entomology. 19: 842-847.

Lindroth, R. L.; Weisbrod, A. V. 1991. **Genetic variation in response of the gypsy moth to aspen phenolic glycosides.** Biochemical Systems and Ecology. 19: 97-103.

Mackauer, M. 1972. **Genetic aspects of insect production.** Entomophaga. 17: 27-48.

Mastro, V. C.; ODell, T. M.; Schwalbe, P. C. 1989. **Genetic control of Lymantriidae: prospects for gypsy moth management.** In: Wallner, W. E.; McManus, K. A., eds. Proceedings, Lymantriidae: a comparison of features of new and old world tussock moths; 1988 June 28-July 1; New Haven, CT. Gen. Tech. Rep. NE-123. Broomall, PA: U.S. Department of Agriculture, Forest Service, Northeastern Forest Experiment Station: 275-302.

Moore, R. F.; ODell, T. M.; Calkins, C. O. 1985. **Quality assessment in laboratory-reared insects.** In: Singh, P.; Moore, R. F., eds. Handbook of insect rearing, vol. I. Amsterdam: Elsevier Press: 107-135.

ODell, T. M. 1993. **Straggling in gypsy moth production strains: a problem analysis for developing research priorities.** In: Anderson, T. A.; Leppla, N. C., eds. Advances in insect rearing for research and pest management. Boulder, CO: Westview Press: 325-350.

ODell, T. M.; Bell, R. A.; Mastro, V. C.; Tanner, J. A.; Kennedy, L. F. 1984. **Production of the gypsy moth, *Lymantria dispar*, for research and biological control.**

In King, E. G.; Leppla, N. C., eds. Advances and challenges in insect rearing. ARS Publ. New Orleans, LA: U.S. Department of Agriculture, Agricultural Research Service: 156-166.

ODell, T. M.; Butt, C. A.; Bridgeforth, A. W. 1985. ***Lymantria dispar.*** In: Singh, P.; Moore, R. F., eds. Handbook of insect rearing, vol. II. Amsterdam: Elsevier Press: 355-367.

Rollinson, W. D.; Lewis, F. B. 1973. **Susceptibility of gypsy moth larvae to *Lymantria* spp. nuclear and cytoplasmic polyhedrosis virus.** Zastita Bilja. 24: 163-168.

Rossiter, M. C. 1987. **Use of a secondary host by non-outbreak populations of the gypsy moth.** Ecology. 68: 857-868.

Rossiter, M. C.; Yendol, G. G.; Dubois, N. R. 1990. **Resistance to *Bacillus thuringiensis* in gypsy moth (Lepidoptera: Lymantriidae): genetic and environmental causes.** Journal of Economic Entomolgy. 83: 2211-2218.

Russell, R. M.; Robertson, J. L.; Savin, N. E. 1977. **POLO: a new computer program for probit analysis.** Bulletin of the Entomological Society of America. 23: 209-215.

Tauber, M. J.; Tauber, C. A.; Roberson, J. R. Tauber, A. J.; Abrahamson, L. P. 1990. **Dormancy in *Lymantria dispar* (Lepidoptera: Lymantriidae), analysis of photoperiodic and thermal responses.** Annals of the Entomological Society of America. 83: 494-503.

Vanderzant, E. S. 1966. **Defined diets for phytophagous insects.** In Smith, C. N., ed. Insect colonization and mass production. New York: Academic Press: 274-303.

Waldvogel, M. G.; Mastro, V. C.; Collinson, C. H.; Cameron, E. A. 1982. **Evaluation of pheromone-mediated responsiveness of laboratory-reared irradiated, laboratory reared non-irradiated and feral male gypsy moths.** Environmental Entomology. 11: 351-354.

Appendix A

The following are pertinent quarterly and annual reports from the APHIS Otis Methods Development Center.

1. Bell, R. A.; Forrester, O. T. 1977. **Gypsy moth mass rearing: evaluation and development of more economical diets and more efficient techniques for rearing and handling gypsy moths.** Progress report, October 1, 1976-March 31, 1977, APHIS Otis Methods Development Center: 58-67.

2. Bell, R. A.; Shapiro, M.; Forrester, O. T. 1976. **Gypsy moth mass rearing: evaluation and development of more economical diets and more efficient techniques for rearing and handling larval gypsy moths.** Progress report, April 1, 1976- September 30, 1976, APHIS Otis Methods Development Center: 54-61.

3. Bell, R. A.; Shapiro, M.; Forrester, O. T. 1977. **Development of diet and efficient rearing techniques.** Progress report, April 1, 1977-September 30, 1977, APHIS Otis Methods Development Center: 77-83.

4. Hansen, R. W. 1988. **Behavioral competitiveness of F_1-sterile gypsy moth adults under field conditions.** Progress report, APHIS Otis Methods Development Center: 173-200.

5. Kennedy, L. F.; Stevens, L. J. 1972. **Rearing large numbers of the gypsy moth, *Porthetria dispar* (L.), under controlled laboratory conditions.** Unpublished report, APHIS Otis Methods Development Center: 1-10.

6. Lance, D. R.; Elkinton, J. S.; Mastro, V. C.; Schwalbe, C. P. 1982. **Behavior and ecology of immature gypsy moths: population quality and competitiveness of F_1-sterile individuals.** Progress report, APHIS Otis Methods Development Center: 76-99.

7. Lance, D. R.; Mastro, V. C.; Elkinton, J. S.; Schwalbe, C. P. 1981. **Behavior and ecology of immature gypsy moths: population quality and competitiveness of F_1-sterile individuals.** Progress report, APHIS Otis Methods Development Center: 148-160.

8. Lance, D. R.; Mastro, V. C.; ODell, T. M.; Shorb, M. D. 1980. **Factors affecting sexual periodicity in male gypsy moths--actograph studies.** Progress report, October 1, 1979-September 30, 1980, APHIS Otis Methods Development Center: 141-151.

9. Lance, D. R.; Mastro, V. C.; Schwalbe, C. P.; ODell, T. M. 1983. **Sterile male technique: studies on the feasibility of releasing F_1-sterile gypsy moth as eggs.** Progress report, APHIS Otis Methods Development Center: 112-119.

10. Mastro, V. C. 1977. **Development and assessment of techniques for comparing the competitiveness of populations of male gypsy moths.** Progress report, May 31, 1977- October 1, 1977, APHIS Otis Methods Development Center: 62-75.

11. Mastro, V. C. 1978. **Assessing male competitiveness traits of various strains of gypsy moth.** Progress report, April 1, 1978-September 30, 1978, APHIS Otis Methods Development Center: 60-71.

12. Mastro, V. C.; Pellegrini-Toole, A.; Lance, D.; Tang, J.; Schwalbe, C. P. 1987. **Competitiveness of gypsy moth adults.** Progress report, APHIS Otis Methods Development Center: 126-139.

13. Mastro, V. C.; Schwalbe, C. P. 1986. **Comparative larval behavior of gypsy moths.** Progress report, APHIS Otis Methods Development Center: 106-110.

14. Paszek, E. 1969. **Rearing conditions--eggs.** Progress report, APHIS Otis Methods Development Center: 11-14.

15. Shapiro, M.; Bell, R. A. 1977. **Gypsy moth mass rearing: evaluation of methods for surface sterilization of eggs and/or pupae.** Progress report, APHIS Otis Methods Development Center: 54-57.

16. Tanner, J. A. 1978. **Establishing standards.** Progress report, October 1, 1977- March 31, 1978, APHIS Otis Methods Development Center: 80-81.

17. Tanner, J. A.; Baker, J. J. 1985. **Evaluating the development and reproduction of insects produced in the Otis Methods Development Center Rearing Facility.** progress report, APHIS Otis Methods Development Center: 117-121.

18. Tanner, J. A.; Baker, J. J.; Fontes, B. A.; Matthews, M., Jr. 1987. **Evaluating the development and reproduction of insects produced in the Otis Methods Development Center Rearing Facility.** Progress report, APHIS Otis Methods Development Center: 146-152.

19. Tanner, J. A.; Baker, J. J.; Hamilton, H. A.; Fontes, B. A. 1986. **Evaluating the development and reproduction of insects produced in the Otis Methods Development Center Rearing Facility.** Progress report, APHIS Otis Methods Development Center: 122-125.

20. Tanner, J. A.; Baker, J. J.; Palmeri, M. 1984. **Evaluating the development and reproduction of insects produced in the Otis Methods Development Center Rearing Facility.** Progress report, APHIS Otis Methods Development Center: 77-79.

21. Tanner, J. A.; Buck, T. 1979. **Establishing standards.** Progress report, October 1, 1978-March 31, 1979, APHIS Otis Methods Development Center: 48-53.

22. Tanner, J. A.; Buck, T. 1979. **Establishing standards.** Progress report, April 1, 1979-September 30, 1979, APHIS Otis Methods Development Center: 101-113.

23. Tanner, J. A.; Demanche, R.; Danville, V. 1984. **The number of instars in the New Jersey Standard Strain.** Progress report, APHIS Otis Methods Development Center: 88-90.

24. Tanner, J. A.; Houle, D. 1978. **Establishing standards.** Progress report, April 1, 1978-September 30, 1978, APHIS Otis Methods Development Center: 82-91.

25. Tanner, J. A.; Lane, S. E.; Taylor, H. M. 1989. **Evaluating the development and reproduction of insects produced in the Otis Methods Development Center Rearing Facility.** Progress report, APHIS Otis Methods Development Center: 119-132.

26. Tanner, J. A.; Tardif, J. G. R.; Baker, J. J.; Fontes, B. A.; Matthews, M., Jr. 1987. **Development and evaluation of improved rearing techniques.** Progress report, APHIS Otis Methods Development Center: 153-167.

27. Tanner, J. A.; Weeks, B. P. 1980. **Establishing standards.** Progress report, APHIS Otis Methods Development Center: 167-175.

28. Tanner, J. A.; Weeks, B. P. 1981. **Evaluating the development and reproduction of insects produced in the Otis Methods Development Center Rearing Facility.** Progress report, APHIS Otis Methods Development Center: 235-247.

29. Tanner, J. A.; Weeks, B. P. 1981. **Development of mechanical egg infestation procedures.** Progress report, APHIS Otis Methods Development Center: 248-256.

30. Tanner, J. A.; Weeks, B. P. 1982. **Evaluating the development and reproduction of insects produced in the Otis Methods Development Center Rearing Facility.** Progress report, APHIS Otis Methods Development Center: 148-152.

31. Tanner, J. A.; Weeks, B. P.; Palmeri, M. 1983. **Evaluating the development and reproduction of insects produced in the Otis Methods Development Center Rearing Facility.** Progress report, APHIS Otis Methods Development Center: 127-135.

Appendix B

Major changes in APHIS-OMDC Rearing Protocol Between F_{13} and F_{20}

Diet. The standard diet now in use evolved from the basic Vanderzant formula to a modified tobacco hornworm diet, to the high wheat-germ or B-4 formula (Bell et al. 1981). As production increased, diet processing stabilized with the use of large food processors. Beginning with F_{20}, diet ingredients for colony production were pre-weighted for the year to ensure that each weekly subcolony was reared on diet containing the same batch (shipment) of ingredients. Pre-weighed ingredients were stored at -15°C (Appendix A, Ref. 28).

Egg Incubation. Treatment of cleaned eggs with sodium hyperchlorite and captan was replaced with a 1-hour soaking of intact or fragmented egg masses in 10 percent formalin followed by a 1-hour rinse in running water and air drying. In the F_{15} generation, experiments indicated that this was the most effective treatment for reducing mortality due to NPV (Appendix A, Ref. 15).

Larval Development. After several changes in containers and attempts to reduce the labor-intensive procedure of transferring larvae to fresh food, a no-transfer system was developed and used starting with the F_{16} generation. In the no-transfer system, a 177.4-ml fluted translucent cup containing 80 ml of diet and fitted with a snap-in paper lid is used. Neonates were transferred to each cup, 8 to 10 per cup, and maintained at 25° ± 1°C, 60 percent RH, and a 17:7 light:dark period (previously 14:10) (Appendix A, Ref. 21).

Pupation, Mating, Oviposition, and Egg Embryonation. Pupae were harvested 35 days after hatch instead of periodically over 2 weeks (Appendix A, Ref. 21). During harvest, pupae were sexed by size and placed in separate containers. They were then placed in 3.8-liter containers, 25 males and 25 females in each, and held for eclosion, mating, oviposition, and egg embryonation at 25° ± 1°C, 60 percent RH, and a 17:7 light:dark period for approximately 42 days (Appendix A, Ref. 21).

Egg Diapause. Egg masses were placed, intact, in cardboard containers (about 100 per container) and transferred to a walk-in chamber. Each container was attached to an automatic mechanical device which carried egg masses through a standard 120-day route. Chamber temperature varies between 5° and 10°C (J. Tanner, pers. commun.). Beginning in F_{20}, time in chill was increased to 170 to 180 days to facilitate more uniform hatch and a shorter incubation time (Appendix A, Ref. 27).

The change to no-larval-transfer, 17 hours of light, single-harvest date (at expense of late developers), multiple mating containers, constant embryonation temperatures, and increased egg storage time and temperature was made primarily to facilitate mass production. Since colony production was not maintained separately, these changes also caused a change in the colony environment.

Infest Method. From F_{20} (October 1982) through F_{34} the only major change in the APHIS-OMDC rearing process occurred in how insects were transferred to the rearing container. In F_{23}, a mechanical egg dispenser that dispensed 12 eggs per container replaced neonate infest (Appendix A, Ref. 29). Eggs were cleaned, sterilized, dried, and placed in the dispenser. Eggs hatched in 3 to 4 days after dispensing. This technique was used for the latter portion of F_{23} through F_{27}. Average percent survival from egg infest to pupation for generations F_{24}-F_{27} ranged from 54.1 (F_{26}) to 64.9 (F_{24}).

Appendix C

Forest Service Rearing Protocol

Diet. The high wheat-germ diet (Bell et al. 1981) is processed weekly in 8-liter batches to provide 90 cups of diet, 80 ml per cup. Ingredients are stored for ≤ 6 months at -6°C.

Egg Incubation. Egg masses are removed from 7° to 8°C storage weekly and sterilized for 1 hour in 10 percent formalin, rinsed for 1 hour, dried, and incubated at 25°C, ≥ 80 percent RH, and a 16:8 light:dark period. After 3 days of incubation, approximately 75 percent of the eggs have hatched and these neonates are used for colony production.

Larval development. Forty-eight neonates from each of 15 egg masses are transferred, 8 per cup, to 177.4-ml fluted translucent plastic rearing cups containing 80 ml of diet. The cups are covered with snap-in cardboard lids and placed on trays in a walk-in environmental chamber at 25°C, 60 percent RH, and a 16:8 light:dark period. The cups are not opened until pupae are harvested 34 days later.

Pupal Harvest, Adult Eclosion, Mating, Oviposition, and Embryonation. Pupae are separated by sex during harvest and mixed between trays to ensure that siblings are not paired. Ten male and 10 female pupae are placed in 0.95-liter containers with a liner of butcher paper. The containers are held in the larval rearing chamber for adult eclosion, mating, and oviposition. After 27 days, dead adults and pupal skins are removed. The butcher paper liners with egg masses are then consolidated, 4 to 5 liners per 0.95-liter container, and maintained at standard rearing conditions for another 15 days. The number of egg masses produced per subcolony ranges from 230 to 316.

Diapause Development. Containers with egg masses are placed in reach-in environmental chambers set at 7° to 8°C, 90 percent RH, and 16:8 light:dark period, and held there for 180 days. Just before incubation a representative number of egg masses from each mating container liner is cut out. There probably is some inadvertent selection for larger, intact egg masses.

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Keena, Melody A.; ODell, Thomas M. 1994. **Effects of laboratory testing on the gypsy moth (Lepidoptera:Lymantriidae)**. Gen. Tech. Rep. NE-181. Radnor, PA: U.S. Department of Agriculture, Forest Service, Northeastern Forest Experiment Station. 23 p.

The history and performance of the New Jersey Standard Strain (NJSS) of the gypsy moth, *Lymantria dispar* (L.), since its establishment in the laboratory in 1967 are reviewed. Phenotypic changes in the NJSS during and after 35 generations of domestication are defined quantitatively and qualitatively. The use of NJSS for research and development should be assessed carefully relative to the reported effects of domestication and the need to equate the performance of NJSS to a specific research/development objective.

Keywords: insecta; gypsy moth; domestication; genetic variability

Headquarters of the Northeastern Forest Experiment Station is in Radnor, Pennsylvania. Field laboratories are maintained at:

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